Table 1. Pearson correlation coefficients

	NTX-I	CTX-II	HA	COMP
Classic TQOL				
K-L 0-IV	0.265*	0.298*	0.302**	0.226*
JSN 0-3	0.229	0.229	0.430***	0.297**
TQOL-Lite				
K-L 0-IV	0.282*	0.321*	0.362***	0.263*
JSN 0-3	0.267*	0.200	0.398***	0.276**

*$p < 0.05$ (2-tailed test). **$p < 0.01$ level (2-tailed test). *$p < 0.001$ level (2-tailed test).

Sum Severity of scores for hips and knees provided similar results to the original TQOL assessment tool, and simplifying its general use. The full TQOL assessment tool should, however, be considered to evaluate biomarker correlations for maximal accuracy in overall assessments.

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CHARACTERISATION OF CARTILAGE TYPE II COLLAGEN DEGRADATION IN THE RABBIT ACLT MODEL

E. Lindhorst[1], L. Wachsmuth[2], N. Kimmig[3], A. Theisen[3], N. Charni[4], F. Juillet[4], P. Garnero[5]
[1]University of Marburg, University of Frankfurt/Main, Marburg, Frankfurt/Main, Germany, [2]University of Erlangen-Nürnberg, Erlangen-Nürnberg, Germany, [3]University of Frankfurt/Main, Frankfurt/Main, Germany, [4]Synarc Molecular Markers, Lyon, France, [5]Synarc Molecular Markers, INSERM Research Unit 664, Lyon, France

Purpose: In humans, one of the most promising biochemical markers of cartilage degradation is the C-terminal crosslinked telopeptide of type II collagen (CTX-II). The rabbit model of anterior cruciate ligament transaction (ACLT) is a valuable animal model of osteoarthritis (OA). The aim of this study was to describe the changes of cartilage degradation by measuring type II collagen degradation fragments (CTX-II) in the urine of rabbits in this ACTL model of OA.
Methods: 2 experimental groups of New Zeland rabbits were studied: ACLT (n=32) and sham operated rabbits (n=32). A medial parapatellar approach was used allowing the transection of the ACL under complete visualisation in the middle third of the ligament with the stumps left in the joint. Sham surgery consisted of a complete arthrotomy including full visualisation of the ACL which was always checked as fully intact. This procedure was performed on only the right knee. At 2, 4, 8 and 12 weeks after surgery, 8 rabbits per experimental group were sacrificed.
A macroscopic grading was performed on the right and left knee joints using the scheme of the International Cartilage Repair Society. Specific areas and sums of areas of all joint sites were analysed. For microscopic evaluation, 4 muem sections of the central tibial plateaus were stained with H&E and Safranin O. Histologic analysis was performed with a dedicated grading system accounting for pathologic alterations of proteoglycan content, matrix structure, cellularity, tidemark duplication, and osteophyte formation, basically a modified Mankin scheme.
Urinary CTX-II was measured by ELISA in 24 hr urine samples, which were obtained during the 24 hrs before surgery and again during 24 hrs each sacrifice time points. Intra- and inter - assay CV was below 10%. Data were corrected for creatinine excretion measured by standard colorimetric assay.
Results: All animals (n = 64) were free of signs of infection and had a normal postoperative course. Lesions advanced with time following the ACLT: at 2 weeks after ACLT, macroscopically visible lesions were only detected at the medial femoral site ($p < 0.05$). With time, lesion size increased and lesions appeared at more joint sites. At 12 weeks after ACLT, medial and lateral tibiae as well as both femoral condyles showed statistically significant lesions. Histologic scores showed early OA after ACLT, when compared with the unoperated contralateral side. Also, a statistically significant difference was seen versus the sham surgery group at the respective timepoints. In sham operated animals, compared to pre-operated controls, urinary CTX-II did not significant changed during the first 8 weeks and then decreased by 65% (p=0.01) at12 weeks. In ACTL rabbits, there was a median 77% increased in CTX-II levels for weeks after surgery and then values decreased.
Conclusions: In this ACLT-rabbit model, urinary CTX-II levels peaked 4 weeks after surgery suggesting that it may be valuable to monitor cartilage degradation in this model of OA.

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CTX II (CARTILAPS®) ASSAY VALIDATION USING EQUINE SYNOVIAL FLUID

T.N. Trumble[1], M.P. Brown[1], K.A. Merritt[1], R.C. Billinghurst[2]
[1]University of Florida, Gainesville, FL, [2]St. Lawrence College, Kingston, ON, Canada

Purpose: The CTX II assay has been used to examine the type II collagen telopeptide concentration in human synovial fluid, explant media, and urine of rats and humans. These reports demonstrate that CTX II may be one of the most beneficial biomarkers for diagnosing, predicting and monitoring changes that occur to the articular cartilage in osteoarthritis (OA) patients. Based on these reports, it seems logical that the CTX II assay has the potential to help diagnose, predict and monitor OA changes in horses since the horse is a good translational model of OA for biomarker evaluation. Type II collagen is well conserved across species, and Nordic Bioscience Diagnostics has demonstrated that the amino acid sequence of the CartiLaps® epitope is 100% conserved across many species including the horse. Thus, the CartiLaps® assay has the potential to be used successfully in equine models of OA.
Methods: Serum Pre-Clinical Cartilaps® ELISAs (Nordic Bioscience Diagnostics) were used for this validation study according to manufacturer protocols. Internal quality control (QC) samples were prepared using the highest concentration standard provided by the manufacturer (259.4 pg/ml). To create QC samples, fresh synovial fluid (SF) was collected aseptically from 6 middle carpal and 6 radiocarpal joints from 3 normal horses. The samples were pooled together for further processing and analysis. Pooled samples were spiked with a known amount of standard to create samples with high, medium and low levels of CTX II. The QC samples were used to determine the precision, specificity, sensitivity, accuracy, linearity of dilution, and stability of this assay with equine SF. To ensure that the assay could detect different biological activity, previously stored SF samples were analyzed from normal rested (n=2) and treadmill exercised horses (n=2), as well as those with naturally-occurring osteochondral (OC) injuries (n=2).
Results: Reproducibility of the standard curve was evaluated (n=6 plates) by computing mean optical density (OD) and percent coefficient of variation (% CV) at each standard concentration. The overall mean inter-assay CV of the standard OD values was 11.1% (range; 6.8-13.7%). Samples exhibited acceptable intra-assay and inter-assay precision over 3 plates with an overall mean CV of 5.2% (range; 0.3-18.8%) and 9.3% (range; 5.3-13.2%), respectively. Parallelism of SF sample dilutions (1:2, 1:4, 1:8, and 1:10) was demonstrated when compared to the standard curve (Figure 1).
Lowest detection limit of the assay was determined to be 6.6 pg/ml. Percent recovery was 64.2% for high, 79.4% for medium, and 93.2% for low QC samples. Linearity of equine SF sample dilutions (1:2, 1:4, 1:8, and 1:16), was demonstrated (Figure